Genetic Variability between Ark Shell (*Scapharca subcrenata*, Lischke) Populations from Daecheon and Wonsan

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ABSTRACT

Genomic DNA isolated from two geographical ark shell (*Scapharca subcrenata*) populations was amplified several times by PCR reactions. The ark shell population from Daecheon (ASPD) and from Wonsan (ASPW) in the West Sea and the East Sea of Korean Peninsula, respectively, obtained. The seven arbitrarily selected primers OPA-05, OPA-11, OPB-09, OPB-11, OPB-14, OPC-18 and OPD-07 were shown to generate the loci observed per primer, shared loci by each population, specific loci, unique shared loci to each population and shared loci by the two populations which could be clearly scored. Here, 862 loci were identified in the ASPD population, and 1,191 in the ASPW population: 137 specific loci (15.9%) in the Daecheon population and 84 (7.1%) in the Wonsan population. 407 shared loci by each population, with an average of 58.1 per primer, were observed in the ASPD population. 473 shared loci by each population, with an average of 67.6 per primer, were identified in the ASPW population. The numbers of specific loci in the ASPD and ASPW population were 137 and 84, respectively. Consequently, the average bandsharing value of individuals within the ASPW population was much higher than in the ASPD population. The bandsharing value between individuals' no. 08 and no. 13 was 0.628, which was the highest measured between the two geographical populations. The dendrogram obtained by the seven primers indicated three genetic clusters: cluster 1 (DAECHEON 01-DAECHEON 11), cluster 2 (WONSAN 12 and 14) and cluster 3 (WONSAN 13, 15, 16, 17, 18, 19, 20, 21 and 22). The genetic distance between the two geographical populations ranged from 0.043 to 0.499. Especially, individual no. 10 of Daecheon population was most distantly related to no. 14 of Wonsan population (genetic distance = 0. 499).

Key words: ark shell, genetic variability, Korean peninsula, *Scapharca subcrenata*, Daecheon, Wonsan.

INTRODUCTION

The ark shell (*Scapharca subcrenata*) is an ecologically and economically important species, belonging to the family Archidae, and the order Arcoida. Ark shell is widely distributed in the entirety of estuary flat habitats, a field of reeds and seawater areas of the West Sea and the southern sea in Korean Peninsula, as well as in several coastal regions of Japan. The mollusks also inhabit in the intertidal depths (approximately 10 m) consisting of a lot of mud, sand and slime. Like other shellfishes basically, the rate at which the shellfish grows depends very much on water temperature. Generally, a water temperature of 20-25℃ is approximately optimal. Ark shells are very thick, heavy and sturdy. The exterior of left shell valve is white or brownish in color. However, the interior color is white like other shellfishes. This shellfish have the sculpture of 30 to 34 radial ribs of square cross-section, each with prominent beads. The left valve of shell is almost as high as long and inequitable valve overlapping right. Left valve of the bivalve is much larger than other right valve. Two valves hinged together dorsally by an elastic, chitinous, external or internal ligament. Basically, the surface color of the shellfish is silvery

Received March 20, 2009; Accepted April 30, 2009
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1225-3480/24313

white and granulated surface of this shell is covered with minute grains or prominent beads but varies widely according to their environment conditions and locality. They also possess hinge with numerous teeth, usually of equal size and perpendicular to main shell axis.

As the ark shell culture industry grows, so doe's interest into the genetics of this shellfish species. It is probable that their number will increase once we have better information on the fisheries and utilization of this group of resources. The seasonal variation of proximate composition, food components, contamination of heavy metals and parasites in various clams have been assessed by various biological methods (Bae, 1998; Park, 2000; Kim *et al.*, 2002; Wang *et al.*, 2005; Wang *et al.*, 2006). However, a little information currently exists regarding the genetics of other shellfish except for granulated ark shell and ark shell from Korea (Kim *et al.*, 2000; Yoon and Kim, 2003a; Jung *et al.*, 2004b). RAPD technique was employed to identify shellfish species such as the blacklip abalone (*Haliotis rubra*) populations (Huang *et al.*, 2000), oyster (*Crassostrea* spp.) (Kim *et al.*, 2004) and purplish Washington clam (*Saxidomus purpuratus*) (Yoon and Park, 2006).

This study was undertaken to determine the genetic distances and differences within and among ark shell populations from Daecheon and Wonsan of Korea. We also analyzed genetic variations and DNA polymorphisms of these two shellfish species of Archidae.

MATERIALS AND METHODS

1. Sample collection, extraction of genomic DNA and amplification stipulations

Two ark shell (*S. subcrenata*) populations were obtained from the Daecheon and Wonsan located in the West Sea and the East Sea of Korean Peninsula. RAPD-PCR analysis was performed on the muscle extracts from 22 individuals, using seven selected decamer primers. The extraction/purification of genomic DNA was performed under the conditions described previously (Yoon and Kim, 2003b). The concentration of the extracted genomic DNA was measured with optical density values at 260 nm, by a spectrophotometer (Beckman Coulter, Buckinghamshire, UK). Seven primers, OPA-05 (5'-AGGGGTCTTG -3'), OPA-11 (5'-CAATCGCCGT -3'), OPB-09 (5'-TGGGGGACTC -3'), OPB-11 (5'-GTAGACCCGT -3'), OPB-14 (5'-TCCGCTCTGG -3'), OPC-18 (5'-TGAGTGGGTG -3') and OPD-07 (5'TTGGCACGGG -3') were shown to generate the loci observed per primer, shared loci by each population, specific loci, unique shared loci to each population and shared loci by the two populations which could be clearly scored. RAPD-PCR was performed using two Programmable DNA Thermal Cyclers (Perkin Elmer Cetus, Norwalk, CT, USA; MJ Research Inc., Waltham, MA, USA). Amplification products were generated via electrophoresis on 1.4% agarose (VentechBio, Korea) gel containing TBE (90 mM Tris, pH 8.5; 90 mM borate; 2.5 mM EDTA). The electrophoresed agarose gels were illuminated by ultraviolet rays, and photographed using a Photoman direct copy system (PECA Products, Beloit, WI, USA).

2. Data analysis

The bandsharing (BS) values were calculated according to Jeffreys and Morton (1987), which is given by the formula: BS = 2 nab / (na+nb), where nab is the number of bands shared between the samples a and b, na is the total number of bands for sample a and nb is the total number of bands for sample b (Jeffreys and Morton, 1987; Yoon and Kim, 2003b; Yoke-Kqueen and Radu, 2006). The average of within-population similarity was calculated by pairwise comparison between individuals within a population. The relatedness between different individuals in the ark shell populations from Daecheon and Wonsan of Korea was generated according to the bandsharing values and similarity matrix. Genetic differences and Euclidean genetic distances within and between populations were calculated using the Systat hierarchical dendrogram program version 10 (SPSS Inc., Chicago, IL, USA). Systat version 10 was also used to obtain other statistical results, including means, standard errors, and t-test scores.

Table 1. The loci observed per primer, shared loci by each population, specific loci, unique shared loci to each population and shared loci by the two populations generated by RAPD-PCR using 7 random primers in ark shell (*S. subcrenata*) from Daecheon and Wonsan of Korea, respectively.

Item	No. of loci observed per lane		No. of shared loci by each population		No. of specific loci	
Primer\Population	Daecheon	Wonsan	Daecheon	Wonsan	Daecheon	Wonsan
OPA-05	158 (14.4)	170 (15.5)	110	88	4	23
OPA-11	126 (11.5)	134 (12.2)	77	66	14	7
OPB-09	144 (13.1)	214 (19.5)	77	110	11	5
OPB-11	143 (13.0)	173 (15.7)	22	44	53	15
OPB-14	100 (9.1)	149 (13.5)	33	22	37	11
OPC-18	115 (10.5)	168 (15.3)	55	66	4	11
OPD-07	76 (6.9)	183 (16.6)	33	77	14	12
Total no.	862 (11.2)	1191 (15.5)	407	473	137	84
Average no. per primer	123.1	170.1	58.1	67.6	19.6	12

The average number of loci generated by a primer in Ark shell obtained Daecheon and Wonsan is shown in parentheses.

RESULTS AND DISCUSSION

1. PCR variations

Genomic DNA was isolated from two geographical ark shell populations in Daecheon and Wonsan. The seven arbitrarily selected primers OPA-05, OPA-11, OPB-09, OPB-11, OPB-14, OPC-18 and OPD-07 were found to generate the loci observed per primer, shared loci by each population, specific loci, unique shared loci to each population (Tables 1 and 2). The number of shared loci by the two populations generated by RAPD-PCR using 7 decamer primers in *S. subcrenata* from Daecheon and Wonsan of Korea (Fig. 4). The size of the DNA loci also varied wildly, from 100 to 2,100 bp (Table 1). In this study, seven oligonucleotide primers generated a total of 862 loci in the Daecheon population, and 1,191 in the Wonsan population, with a DNA loci size ranging from 100 to 2,100 bp, as summarized in Tables 1 and 2.

Table 2. The number of unique shared loci to each species generated by PCR using 7 decamer primers in *S. subcrenata* from Daecheon and Wonsan of Korea.

Item	No. of unique shared loci to each population	
Primer \ Population	Daecheon	Wonsan
OPA-05	110	88
OPA-11	22	11
OPB-09	44	77
OPB-11	0	22
OPB-14	22	11
OPC-18	55	66
OPD-07	11	55
Total no.	264	330
Average no. per primer	37.7	47.1

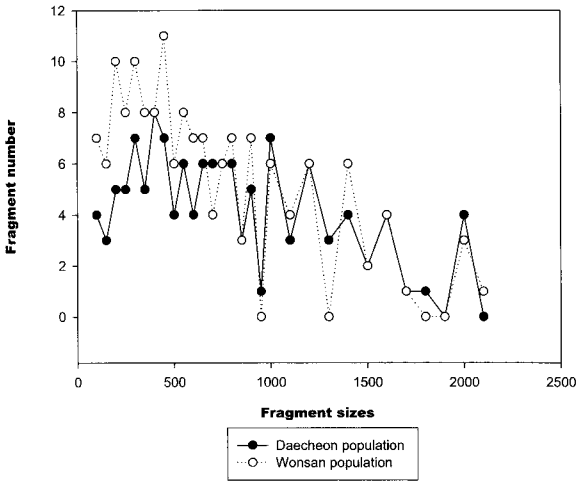

Fig. 1. Distribution of fragment sizes of two ark shell populations. Circles: Daecheon ark shell population. Blank circles: Wonsan ark shell population. The fragment numbers in each size interval have been computed from the pooled fragments obtained with all the primers.

Table 3. Similarity matrix, including bandsharing values and genetic differences, calculated using Nei and Li's index, of the similarity of ark shell (*S. subcrenata*) from Daecheon and Wonsan, respectively.

	Bandsharing values of ark shell																					
	from Daecheon (1-11)										from Wonsan (12-22)											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	-	0.838	0.815	0.798	0.748	0.820	0.764	0.747	0.786	0.683	0.738	0.549	0.558	0.495	0.538	0.528	0.501	0.511	0.501	0.544	0.540	0.548
2		-	0.803	0.825	0.781	0.785	0.753	0.760	0.734	0.718	0.713	0.543	0.538	0.536	0.556	0.577	0.542	0.554	0.506	0.581	0.537	0.571
3			-	0.775	0.748	0.752	0.729	0.746	0.723	0.722	0.752	0.533	0.546	0.505	0.572	0.549	0.531	0.540	0.508	0.559	0.531	0.544
4				-	0.781	0.768	0.797	0.808	0.821	0.755	0.771	0.553	0.584	0.548	0.605	0.602	0.591	0.614	0.557	0.599	0.558	0.613
5					-	0.769	0.783	0.765	0.685	0.842	0.750	0.548	0.584	0.527	0.593	0.558	0.553	0.564	0.530	0.579	0.539	0.569
6						-	0.728	0.720	0.725	0.696	0.708	0.509	0.531	0.479	0.533	0.534	0.516	0.531	0.494	0.545	0.508	0.541
7							-	0.782	0.779	0.730	0.773	0.501	0.535	0.456	0.520	0.551	0.525	0.536	0.515	0.562	0.514	0.519
8								-	0.792	0.731	0.783	0.552	0.628	0.522	0.608	0.576	0.577	0.576	0.540	0.568	0.524	0.576
9									-	0.745	0.751	0.531	0.546	0.458	0.531	0.554	0.566	0.556	0.529	0.525	0.499	0.501
10										-	0.703	0.565	0.546	0.497	0.535	0.556	0.536	0.539	0.540	0.590	0.565	0.573
11											-	0.582	0.562	0.505	0.542	0.541	0.554	0.521	0.531	0.538	0.504	0.565
12												-	0.722	0.718	0.787	0.764	0.751	0.725	0.734	0.716	0.686	0.724
13													-	0.824	0.879	0.827	0.848	0.863	0.858	0.767	0.798	0.836
14														-	0.839	0.792	0.834	0.817	0.813	0.776	0.715	0.797
15															-	0.882	0.877	0.888	0.865	0.793	0.832	0.861
16																-	0.891	0.910	0.904	0.776	0.823	0.869
17																	-	0.898	0.868	0.792	0.788	0.830
18																		-	0.917	0.791	0.826	0.840
19																			-	0.775	0.817	0.892
20																				-	0.830	0.791
21																					-	0.853
22																						-

Researchers have studied the sizes of DNA fragments in the RAPD-PCR profiles of Eastern Pacific abalone (genus *Haliotis*) (Muchmore *et al.*, 1998) and marsh clam (*Corbicula* spp.) (Yoon and Kim, 2003a). The various sizes of the DNA loci ranging from 50 to 2,400 bp were generated in two geographical purplish Washington clam (*Saxidomus purpuratus*) populations in Gunsan and Haeju (Yoon and Park, 2006).

We assessed genetic variation in the Daecheon population first. Primer OPA-05 detected 110 shared loci by each population major and/or minor loci of sizes 100 bp and 1,200 bp, which were identical in all samples (Table 1) (Fig. 1A). This primer generated the most loci, a total of 159, although the average was 14.4. One specific major locus (approximately 600 bp; lane 4) and three minor loci (200 bp; lanes 1, 2 and 10) were also detected. The 22 shared loci by each population obtained with the decamer primer OPB-11, approximately 250 bp and 800 bp in size, were observed in all samples (Fig. 2D). The 53 specific loci generated by this primer exhibited inter-individual-specific characteristics, thus revealing DNA polymorphisms. The primer OPB-14 generated 33 shared loci by each population in all samples, of approximately 300 bp, 350 bp and 1,000 bp in size (Fig. 2E). This primer produced an average of 9.1 loci per lane, and a total of 100 loci, in comparison to the other primers used. The random primer OPC-18 detected 33 shared loci by each population of 400 bp, 550 bp, 750 bp, 850 bp, 1,200 bp and 1,600 bp in all samples (Fig. 2F). Interestingly, four specific loci that established the identity of an individual was 150 bp

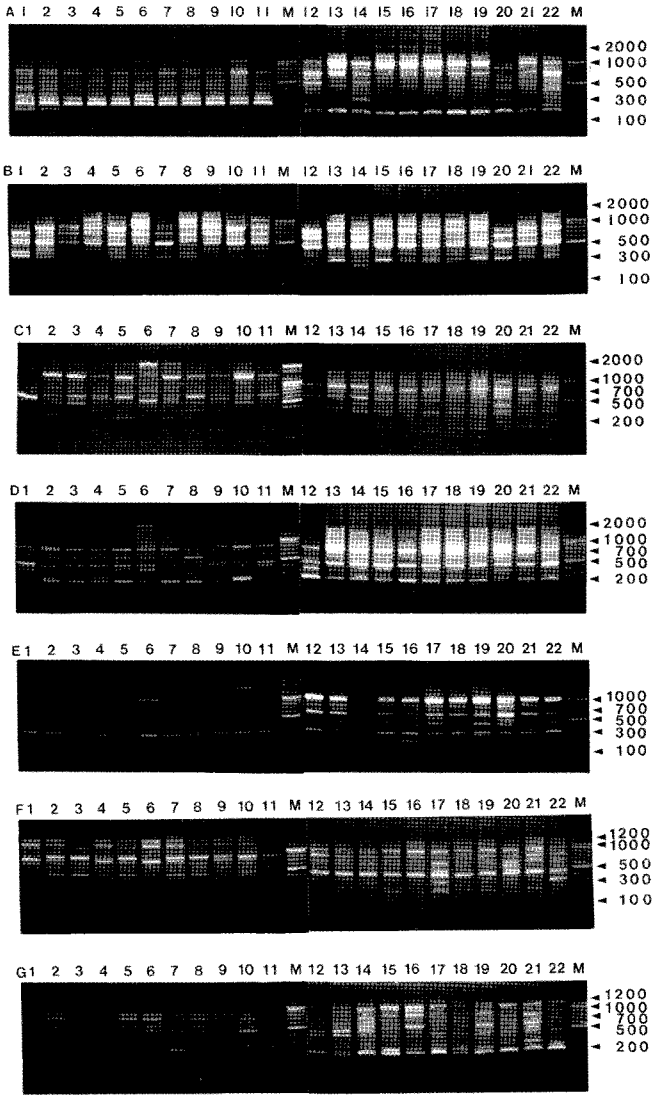

Fig. 2. PCR-based electrophoretic outlines of individual ark shell (*S. subcrenata*). Genomic DNAs isolated ark shell (lane 1-11) from Daecheon in the West Sea and ark shell (lane 12-22) from Wonsan in the East Sea, respectively, were amplified by random primers OPA-05 (A), OPA-11 (B), OPB-09 (C), OPB-11 (D), OPB-14 (E), OPC-18 (F) and OPD-07 (G). Amplified products were electrophoresed on 1.4% agarose gel and detected by staining with ethidium bromide. Each lane shows DNA samples extracted from 22 individuals. M: 100 bp step ladder marker.

(lane 11), 250 bp (lanes 9 and 11) and 1,100 bp (lane 3). The primer OPD-07 detected 33 shared loci by each population major and/or minor of sizes 550 bp, 700 bp and 800 bp, in all samples (Table 1) (Fig. 2G). This primer produced the least of loci per lane (a total of 76), in comparison to the other primers used, with an average of 6.9. The complexity of the banding patterns varied widely between primers and/or geographic locales. Generally, the size and number of fragments generated depends both on the nucleotide sequence of the primer used, and on the source of the template DNA, resulting in a genome-specific DNA fragment (Welsh and McClelland, 1990; Welsh *et al.*, 1991).

Moreover, in the Wonsan ark shell population, genetic variation was identified in the banding pattern generated by decamer OPB-14, which ranged from approximately 100 bp to 1,500 bp, as illustrated in Table 1 (Fig. 2E). This primer generated 149 loci per lane, with an average of 13.5. The 22 shared loci by each population, of approximately 300 bp and 450 bp, represented the geographical population. RAPD variation was observed in the banding patterns, ranging from 100 bp to 1,600 bp, and was generated by the decamer primer, OPB-09 (Fig. 2C). This primer generated the most loci (a total of 214), with an average of 19.5, as described in Table 1. This decamer primer generated 5 specific loci, of approximately 250 bp (lanes 16, 20 and 22) and 450 bp in size (lanes 19 and 22). A high degree of RAPD variation was observed in the banding patterns, ranging from 100 bp to 2,100 bp, and was generated by the decamer primer, OPA-05. 88 shared loci by each population were identified, which established the identifications for populations and/or species (Fig. 2A). This primer generated a specific RAPD profile consisting of 23 DNA fragments. This primer also generated an average of 15.5 loci, and a total of 170 loci per lane.

3. Variation within and between populations, and genetic distances

In this study, several specific loci generated by the seven decamer primer also exhibited inter-individual-specific characteristics and DNA polymorphisms, as shown in Fig. 2. Here, 862 loci were identified in the Daecheon population, and 1,191 in the Wonsan population: 137 specific loci (15.9%) in the Daecheon population and 84 (7.1%) in the Wonsan population (Table 1). These specific primers proved useful in the identification of individuals and/or populations, resulting from variations in DNA polymorphisms among individuals/populations (Liu *et al.*, 1998; Yoon and Park, 2002; Yoon and Kim, 2003b; Yoon and Kim, 2004). We identified a great deal of specific loci like this and the result of this analysis demonstrate unique individuals exist abundantly in the ASPD population. Here, we was able to identify the similar homogeneity between two ark shell populations as we

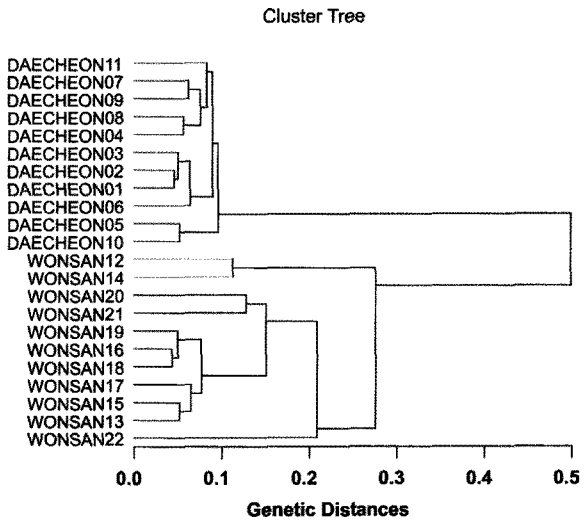

Fig. 3. Hierarchical dendrogram of genetic distances, obtained from two geographical populations of ark shell (*S. subcrenata*). The relatedness between different individuals in the ark shell populations from Daecheon and Wonsan of Korea, were generated according to the bandsharing values and similarity matrix (see Table 3).

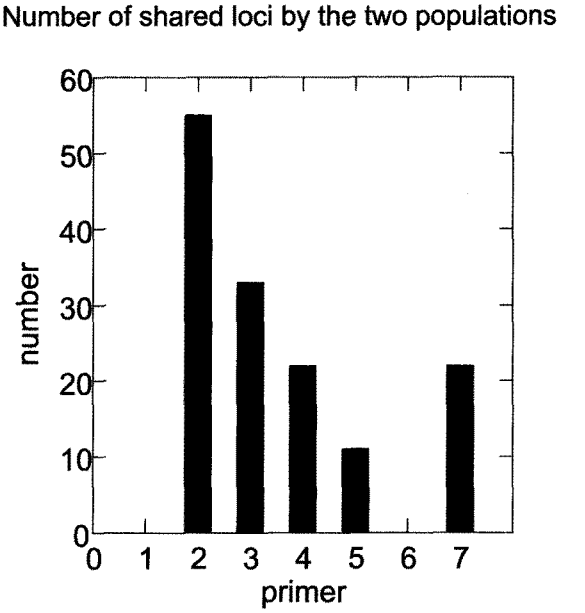

Fig. 4. The number of shared loci by the two populations generated by RAPD-PCR using 7 decamer primers in *S. subcrenata* from Daecheon and Wonsan of Korea. 1: OPA-05, 2: OPA-11, 3: OPB-09, 4: OPB-11, 5: OPB-14, 6: OPC-18, 7: OPD-07. The total number and average number of shared loci by the two populations generated by a primer in ark shell obtained Daecheon and Wonsan showed 143 and 20.4, respectively.

considered the number of shared loci, as illustrated in Table 1.

407 shared loci by each population, with an average of 58.1 per primer, were observed in the Daecheon population. 473 shared loci by each population, with an average of 67.6 per primer, were identified in the Wonsan population. The numbers of specific loci in the Daecheon and Wonsan population were 137 and 84, respectively. The decamer primer, OPA-11, generated shared loci by the two populations, of approximately 300 bp, 400 bp, 500 bp, 800 bp and 1,000 bp, respectively, in both the Daecheon and Wonsan populations (Fig. 2B) (Fig. 4). The decamer primer, OPD-07, also generated shared loci by the two populations, of approximately 700 bp and 800 bp each, in the Daecheon and Wonsan populations (Fig. 2G) (Fig. 4).

Founded on the average bandsharing values of all samples, the similarity matrix ranged from 0.683 to 0.842 in the Daecheon population, and from 0.686 to 0.917 in the Wonsan population (Table 3). The average bandsharing value was 0.760 ± 0.005 within the Daecheon population, and 0.816 ± 0.008 within the Wonsan population. The average bandsharing value between the two geographical ark shell populations was 0.544 ± 0.003, ranging from 0.456 to 0.628. The bandsharing value between individuals no. 05 and no. 10 was 0.842, which was the highest value identified within the Daecheon population. The bandsharing value between individuals' no. 01 and no. 10 was 0.683, which was the lowest observed. The bandsharing value between no. 18 and no. 19 was 0.917, which was the highest value observed within the Wonsan population. Consequently, the average bandsharing value of individuals within the Wonsan population was much higher than in the Daecheon population. The bandsharing value between individuals' no. 08 and no. 13 was 0.628, which was the highest measured between the two geographical populations. The value between individuals' no. 07 and no. 14 was 0.456, which was the lowest seen between the two geographical populations. Accordingly, as stated above, RAPD-PCR analysis indicated that the ark shell population from Daecheon was more genetically diverse than the Wonsan ark shell population. Our reported bandsharing values between the two geographical ark shell populations are inconsistent with previously reported results (Yoon and Park 2002). The average bandsharing value recorded in our study is higher than the average value between the two oyster populations (0.282 ± 0.008) (Kim *et al.*, 2004). However, the average bandsharing value reported by our study is lower than the value reported for Spanish barbel species (0.71-0.81) (Callejas and Ochando, 1998).

To obtain the dendrogram, we carried out a hierarchical clustering analysis, utilizing the similarity matrix based on the bandsharing values and genetic differences (Fig. 3). The dendrogram obtained by the seven primers indicated three genetic clusters: cluster 1 (DAECHEON 01-DAECHEON 11), cluster 2 (WONSAN 12 and 14) and cluster 3 (WONSAN 13, 15, 16, 17, 18, 19, 20, 21 and 22). The genetic distance between the two geographical populations ranged from 0.043 to 0.499. The shortest genetic distance representing a significant molecular difference was between individuals' no. Wonsan 18 and no. 16 of Wonsan (0.043). Individual no. 10 of Daecheon population was most distantly related to no. 14 of Wonsan population (genetic distance = 0. 499). Individual no. 14 from Wonsan was more or less genetically-distantly related to Wonsan no. 12 (genetic distance = 0.113). In particular, the longest genetic distance representing significant molecular differences, 0.182, was found to exist between individual's no. 03 of Daecheon and no. 11 of Wonsan. Our cluster analysis revealed a pattern similar to the one posited by Yoon and Kim (2004). They reported that single linkage cluster analysis, which indicated four genetic groupings, and the dendrogram revealed close relationships between individual identities within two geographical populations.

In fish and invertebrates, cluster analysis of the pairwise population matrix, generated from RAPD data, showed that geographically close populations tended to cluster together in the blacklip abalone (Huang *et al.*, 2000). This study showed that large genetic differences could be found between

geographical populations within a species, as well as between species. Phylogenetic relationships among 5 *Haliotis* species and one hybrid were conducted by calculation of the distance coefficient and construction of a phylogenetic tree based on RAPD data (Kim *et al.*, 2000). They insisted that RAPD analysis constitutes a powerful tool for the elucidation of phylogenetic relationships, based on their analysis of 6 species of Haliotis. The dendrogram obtained from the Korean oyster population by the four primers, indicates three genetic clusters (Kim *et al.*, 2004). The genetic distance between the two geographic populations ranged from 0.039 to 0.284. The shortest genetic distance representing significant molecular differences, 0.080, was found to exist between individuals no. 09 and no. 07 from Buan.

The identification of the penaeid shrimp (*Penaeus chinensis*), bullhead (*Pseudobagrus fulvidraco*), and oyster (*Crassostrea gigas*) populations constituted a necessary step in the inception and development of invertebrate/teleost breeding programs (Yoon and Kim 2003b; Yoon and Kim, 2004; Kim *et al.*, 2004). The classification of geographical populations of ark shell is based on morphological variations in head type, body size, body type, body color, fin type, and eye type. It is assumed that differences in such traits reflect distinct origins or genetic identity (Chenyambuga *et al.*, 2004). As stated above, the potential of RAPD to identify diagnostic markers for breed, stock, species and population identification in teleosts (Callejas and Ochando, 1998; Yoon and Kim, 2004), and in shellfish (Tassanakajon *et al.*, 1998; Yoon and Kim, 2003b; Kim *et al.*, 2004) has also been well established. In our study, RAPD-PCR analysis has revealed a significant genetic distance between two population pairs. RAPD-PCR enabled us to detect the existence of population discrimination and genetic variation in ark shell populations of Daecheon and Wonsan. This confirms that the method is a suitable tool for DNA comparisons, both within and between individuals, species, and populations. Furthermore, basic knowledge of the DNA polymorphisms and molecular markers in ark shell (*S. subcrenata*) may contribute significantly to broodstock selection and selective fish-breeding programs. The extraordinarily unique gene pools exhibited by some samples (especially in the case of the photo in Fig. 2F) would require new conservation policies, such that many wilder Korean ark shell populations could be preserved.

ACKNOWLEDGEMENTS

We express our gratitude to Dr. Yong-Ho Kim from the Department of Marine Aquaculture and Biotechnology and colleagues from Physiology & Genetics Laboratory of the Department of Aquatic Life Medicine, Kunsan National University, for their assistance in sample collection, and their help with the RAPD-PCR techniques throughout this study. The authors also would like to thank the reviewers who assisted us with thorough and profound correction. The authors wish to acknowledge the financial support of the Fisheries Science Institute of Kunsan National University in the program year of 2009.

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